Journal of Environmental Science and Health, Part B
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597269

Effect of nitroethane and nitroethanol on the production of indole and 3-methylindole (skatole) from bacteria in swine feces by gas chromatography

Ross C. Beier [a]; Robin C. Anderson [a]; Nathan A. Krueger [a]; Thomas S. Edrington [a]; Todd R. Callaway [a]; David J. Nisbet [a]
[a] U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, Texas, USA

Online Publication Date: 01 August 2009

To cite this Article Beier, Ross C., Anderson, Robin C., Krueger, Nathan A., Edrington, Thomas S., Callaway, Todd R. and Nisbet, David J.(2009)'Effect of nitroethane and nitroethanol on the production of indole and 3-methylindole (skatole) from bacteria in swine feces by gas chromatography',Journal of Environmental Science and Health, Part B,44:6,613 — 620

To link to this Article: DOI: 10.1080/03601230903000701

URL: http://dx.doi.org/10.1080/03601230903000701

Journal of Environmental Science and Health Part B (2009) **44**, 613–620
ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601230903000701

Taylor & Francis
Taylor & Francis Group

Effect of nitroethane and nitroethanol on the production of indole and 3-methylindole (skatole) from bacteria in swine feces by gas chromatography

ROSS C. BEIER, ROBIN C. ANDERSON, NATHAN A. KRUEGER, THOMAS S. EDRINGTON, TODD R. CALLAWAY and DAVID J. NISBET

U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, Texas, USA

Indole and 3-methylindole (skatole) are odor pollutants in livestock waste, and skatole is a major component of boar taint. Skatole causes pulmonary edema and emphysema in ruminants and causes damage to lung Clara cells in animals and humans. A gas chromatographic method that originally used a nitrogen–phosphorus detector to increase sensitivity was modified resulting in an improved flame ionization detection response for indole and skatole of 236% and 207%, respectively. The improved method eliminates the large amount of indole decomposition in the injector. A 10 μg mL^{-1} spike of indole and skatole in water and swine fecal slurries resulted in recovery of 78.5% and 96% in water and 76.1% and 85.8% in fecal slurries, respectively. The effect of the addition of nitroethane and nitroethanol at 21.8 mM in swine fecal slurries was studied on the microbial production of indole and skatole. Nitroethane and nitroethanol decreased the production of skatole in swine fecal slurries at 24 h. The nitroethane effect on L-tryptophan-supplemented fecal slurries after 6 and 24 h incubation resulted in a decrease of 69.0% ($P = 0.02$) and 23.5% skatole production, respectively, and a decrease of 14.9% indole at 6 h, but an increase in indole production of 81.1% at 24 h.

Keywords: Boar taint; fecal bacteria; indole; metabolism; nitroethane; nitroethanol; pulmonary edema; skatole; swine feces; tryptophan.

Introduction

Sweeping changes have been made in livestock production over recent years that have resulted in intensive production techniques generating large amounts of liquid manures.[1] The storage and land application of livestock manure can cause an odor nuisance, which may result in the reduction in value of neighboring properties.[2] Various odorants have been identified as being important contributors to livestock odor nuisance.[3] Commonly, ammonia and hydrogen sulfide emissions are evaluated (for example[4]). However, indole and 3-methylindole (skatole) (Fig. 1, A and B) have also been identified in the odorous emissions from pig slurries.[2,3,5] Skatole was one of the major volatile components in pig slurry odor and was second in quantity to 4-methylphenol in a pig slurry diethyl ether extract.[3] High levels of skatole in the fat of male pigs along with high levels of the steroid pheromone, androstenone, is the major cause of boar taint, the unpleasant flavor associated with meat from male pigs.[6–11] Indole and skatole are the main products of bacterial L-tryptophan metabolism,[12] and maybe as important, indole and skatole are produced from L-tryptophan by bacteria in bovine rumen.[13,14]

Intraruminal doses of L-tryptophan caused acute pulmonary edema and emphysema in cattle.[15–17] The L-tryptophan-induced pulmonary edema and emphysema in cattle was associated with ruminal and plasma concentrations of skatole, and severity of the pulmonary lesions were related to the concentration of skatole found in the plasma.[18] Skatole has been associated with a number of disease conditions in various animal species. Pulmonary edema and emphysema was demonstrated in cattle following intraruminal and intravenous administration of skatole[19,20] and in goats after oral doses of skatole.[20] Oral or intravenous administration of skatole causes destruction

Address correspondence to Ross C. Beier, U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, 2881 F&B Road, College Station, TX 77845-4988, USA; E-mail: ross.beier@ars.usda.gov
Mention of trade names, proprietary products, or specific equipment is solely for the purpose of providing specific information and does not constitute a guarantee, warranty or endorsement by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.
Received February 23, 2009.

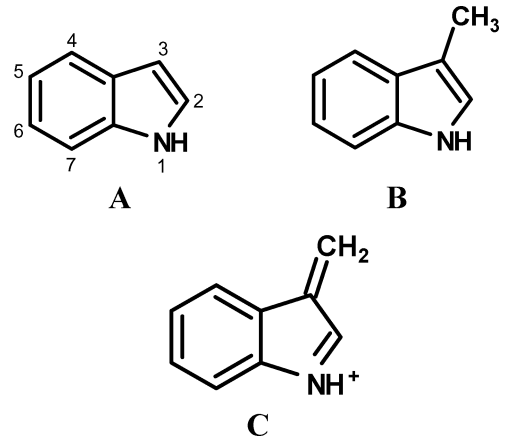

Fig. 1. Chemical structures of (A) indole, (B) 3-methylindole (skatole), and (C) 3-methyleneindolenine (the reactive intermediate causing pneumotoxicity).

of lung Clara cells in goats[21,22] and sheep[23] as well as selective damage to lung Clara cells in the horse,[24] rat and mouse.[25] Skatole also causes olfactory mucosal injury in the mouse,[26] disruption of axonal transport between the olfactory epithelium and olfactory bulb in the rat,[27,28] and olfactory bulb deafferentation in the rat.[29]

The toxicity is caused by a transient free radical[30] produced by cytochrome *P*-450 bioactivation of skatole.[31] The toxic intermediate from bioactivated skatole has been shown to be the methylene imine, 3-methyleneindolenine (Fig. 1, C),[32] which covalently binds proteins.[32,33] Bioactivation of skatole resulting in toxicity is similar to the cytochrome *P*-450 bioactivation of two other toxic natural products. The skatole-induced pulmonary lesions in mice[26] resemble the sweet potato lung toxin, 4-ipomeanol, induced lesions in rodents.[34] Cytochrome *P*-450 bioactivates 4-ipomeanol in lung Clara cells, the reactive-intermediate readily covalently binds proteins, and is known to cause lung edema in cattle.[35] Goats are the most susceptible animal to skatole toxicity,[20,32] and goats are also very susceptible to white snakeroot toxicity.[36] White snakeroot contains tremetone that can be bioactivated by cytochrome *P*-450 resulting in cell killing in cell culture.[37,38] But white snakeroot results in general tissue toxicity, and not only primarily in lung tissue.[36]

Humans may be susceptible to skatole-mediated toxicities.[39] Human bronchial epithelial cells bioactivate skatole to produce 3-methyleneindolenine, which induces programmed cell death at low skatole concentrations.[40] Humans may have additional routes of exposure to skatole; cheeses,[41] cigarette smoke,[42–46] coffee,[47] seafood,[48] and tryptophan metabolism by intestinal microflora.[49] But there may be substantial exposure from animal waste storage pits, holding reservoirs or lagoons, especially for farm workers. Donham et al. found that 87% of swine confinement workers reported work-related cough,[50] which agreed with previous studies that swine confinement workers experience significant insult to the respiratory tract resulting in bronchitis and reactive airways. Neighbors of a large-scale swine operation reported experiencing higher rates of symptoms associated with respiratory inflammation than did demographically similar subjects living near minimal livestock production.[51] Environmental odors emanating from large-scale hog operations may also have a pronounced effect on mood and stress levels.[52] Previous observations suggested that nitroethane and nitroethanol may reduce the odor of swine feces (R.C.A. personal communication). To investigate ways of altering microbial degradation of L-tryptophan in fecal slurries, it was necessary to develop a simple, accurate method to quantify indole and skatole.

Various methods have been used to quantify indole and skatole. Skatole has been quantified from pig backfat by gas chromatography (GC),[53,54] high-performance liquid chromatography (HPLC),[55] and spectrophotometry methods.[8,56] HPLC was used to quantify indole and skatole in swine gut contents,[57] and to quantify skatole in various animal feces.[58] A GC-mass selective detector (MSD) was used to determine the emission rates of indole, skatole, and other odorous compounds from pig fecal slurries.[5] GC-mass spectrometry (MS) was used to quantify indole and skatole in the volatile compounds from poultry manure,[59] in pig and chicken waste odors,[3] and in emissions of swine waste lagoons.[60] GC has been used to quantify indole and skatole from bovine plasma[61] and ruminal fluid.[17] A GC method using a nitrogen–phosphorus detector to improved sensitivity was used to quantify indole and skatole from culture media, intestinal contents, feces,[62] and in pig fecal slurries.[63]

The initial goal of the current study was to develop a simple GC method for the quantification of indole and skatole in pig fecal slurries. The conditions used by Jensen and Jensen[62,63] were studied to determine the source of poor sensitivity that was obtained for indole and skatole during extraction and GC analysis that required the use of a nitrogen–phosphorus-sensitive detector. In this study, a previous method[62] was modified resulting in an improved flame ionization detector (FID) response. The second goal was to use this new GC-FID method to evaluate the effect of nitroethane and nitroethanol on the production of indole and skatole in swine fecal slurries.

Materials and methods

Chemicals and materials

Indole, 5-methylindole, NaCl, nitroethane, nitroethanol, skatole, and 200 proof anhydrous EtOH were obtained from Sigma–Aldrich (Milwaukee, WI). L-Tryptophan was obtained from Sigma–Aldrich (St. Louis, MO). Hexanes (boiling range 68–70°C) was obtained from EM Science (Gibbstown, NJ). Five-mL sterile microtubes

(No. 80078-650) were obtained from VWR International (West Chester, PA). Whirl-packs were obtained from BVA Scientific (San Antonio, TX). H_2O was produced on site by a reverse osmosis system obtained from Millipore Corp. (Bedford, MA).

Sample collection

Swine feces were collected from market aged feeder pigs immediately following voiding. The collected feces was placed in whirl-packs and delivered to the laboratory (adjacent to the farm site). A swine fecal slurry was then generated by making a 1:5 mixture of swine feces and an anaerobic dilution solution, respectively, according to Bryant and Burkey.[64] The feeder pigs were raised according to standard husbandry practices as defined by our Institutional Animal Care and Use Guidelines, No. 2007001.

Instrumentation

The Jensen and Jensen[62] gas chromatographic method was modified by using a 25 m × 0.32 mm ID, BP20 (0.5 μm film thickness) capillary column (SGE Inc., Austin, TX) on a HP 6890 GC System (Agilent Technologies, Santa Clara, CA) with FID peak detection. Hydrogen was used as the carrier gas at 2.0 mL min^{-1}. The mode was splitless and the injector temperature was set at 250°C with an initial column temperature of 80°C. A double ramp temperature program was used at 16°C min^{-1} to 185°C, hold for 6.25 min, followed by 20°C min^{-1} to 240°C, with a final hold for 16.3 min. The Hermle Z230A centrifuge was obtained from National Labnet Co. (Woodbridge, NJ).

Analytical procedures

Preparation of standards

Indole and skatole standards (200 μg mL^{-1}) used for producing the standard curve were made in hexanes (boiling range 68–70°C). The 5-methylindole internal standard (ISD) (200 μg mL^{-1}) was made in 200 proof anhydrous EtOH. Standard curves were prepared for indole and skatole by adding quantities of indole and skatole standards in hexanes with 30 μL of the ISD solution plus hexanes solvent to produce a concentration range of 0.5–90 μg mL^{-1} indole and skatole with a concentration of 6 μg mL^{-1} ISD. Indole and skatole 200 μg mL^{-1} standards used for spiking water samples and fecal slurries were made in 200 proof anhydrous EtOH.

Injector temperature experiment

Indole and skatole samples in hexanes solvent (10 μg mL^{-1}) were injected into a 250°C injector, and the FID response in pA was used for comparison to indole and skatole samples in chloroform solvent (10 μg mL^{-1}) injected into a 150, 200, 250, 275, and 300°C injector.

Extraction method

Samples were quantified for indole and skatole using the following extraction method: To a 1 mL water spiked or fecal slurry sample, a 1 mL NaCl solution (34 g NaCl per 100 mL H_2O) was added in a 5-mL sterile microtube. To this solution 50 μL of 4N NaOH and 30 μL of ISD (6 μg mL^{-1} final concentration) were added. Finally, 1 mL of hexanes was added and the sample was vortexed until an emulsion formed, and then centrifuged for 10 min at 2,739 × g in a Hermle Z230A centrifuge. The hexanes layer was then transferred to a sample vial and a 2 μL sample was injected into the GC.

Water and fecal slurry spiking experiment

Water was spiked with indole and skatole EtOH standards resulting in a final concentration of 10 μg mL^{-1}. These spiked water samples were then extracted and analyzed by GC according to the above methods. The swine fecal slurry was spiked with indole and skatole standards in EtOH resulting in a final concentration of 10 μg mL^{-1} of either compound. These spiked fecal slurry samples were also extracted and analyzed by GC according to the above methods.

Nitroethane and nitroethanol effect on indole and skatole production

The swine fecal slurry was anaerobically (under 100% CO_2) distributed in 12 mL volumes. Nitroethane or nitroethanol (0.1 mL, 2664 mM in H_2O) was anaerobically added to the appropriate sample tubes (final concentration = 21.8 mM) with and without L-tryptophan (0.1 mL, 24 mg mL^{-1} in H_2O). H_2O (0.1 or 0.2 mL) was added anaerobically to the appropriate tubes to make the mixtures have equal volume. Supplemented fecal slurries and other controls were kept anaerobically until samples (1 mL) were processed according to the extraction method above at 0.5, 6, and 24 h, and then indole and skatole were quantified according to the above GC method.

Data analysis

Concentrations of indole and skatole during in vitro incubations were analyzed for treatment differences by a repeated measures analysis of variance using Statistix8 Analytical Software (Tallahassee, FL). Means were separated using a Tukey's procedure.

Results and discussion

The analysis of indole and skatole (Fig. 1, A and B) required a simple, effective analysis method. The method of Jensen and Jensen[62] initially appeared to be appropriate; however, it was quickly realized that the method resulted in extremely poor sensitivity for indole and skatole using FID. Thus the method[62] was investigated to determine if it

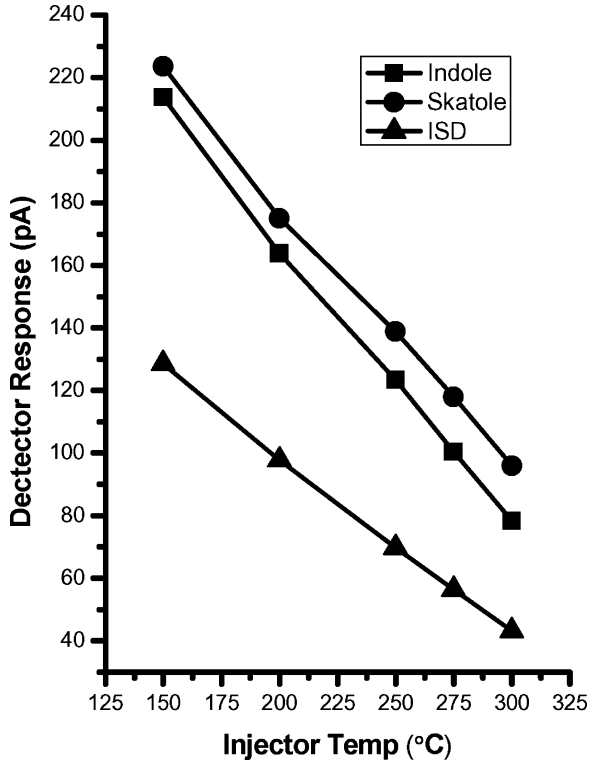

Fig. 2. Detector response for indole, skatole, and the internal standard (ISD) with $CHCl_3$ as the injection solvent at injector temperatures of 150–300 °C.

might be modified to increase the sensitivity using FID. The original method used chloroform for indole and skatole extraction and as injection solvent in the GC at an injector temperature of 300°C. Figure 2 shows the results of injecting a 10 μg mL^{-1} chloroform solution of indole and skatole containing the ISD 5-methylindole in the GC with injector temperatures ranging from 150–300°C. Detector responses for all three chemicals were drastically reduced as the injector temperature increased to 300°C. This loss in sensitivity using chloroform as solvent led to Jensen and Jensen[62] requiring the use of a nitrogen–phosphorus-sensitive detector, but the large decomposition of these indoles in the injector could alter the value of the results.

To improve the sensitivity of the Jensen and Jensen method, the method was modified in three ways: a) hexanes (boiling range 68–70°C) was used instead of chloroform for extraction and injection solvent, b) the injector temperature was lowered to 250°C, and c) a salt solution was added during extraction instead of only H_2O. These changes resulted in an FID response for indole, skatole, and ISD of 185.1 ± 2.8, 198.4 ± 2.9, and 117.5 ± 1.8 pA, respectively. In comparison with the method of Jensen and Jensen[62] (Fig. 2, 300°C injector temperature with chloroform solvent), the detector response for indole, skatole, and the ISD using the modified method increased the FID response by 236, 207, and 272%, respectively, and provided sufficient sensitivity for quantification of indole and skatole. Standard curves for indole and skatole with the new modified method had a correlation of r = 0.99984 and 0.99988, respectively. A 10 μg mL^{-1} spike of indole and skatole in water and a fecal slurry is shown in comparison

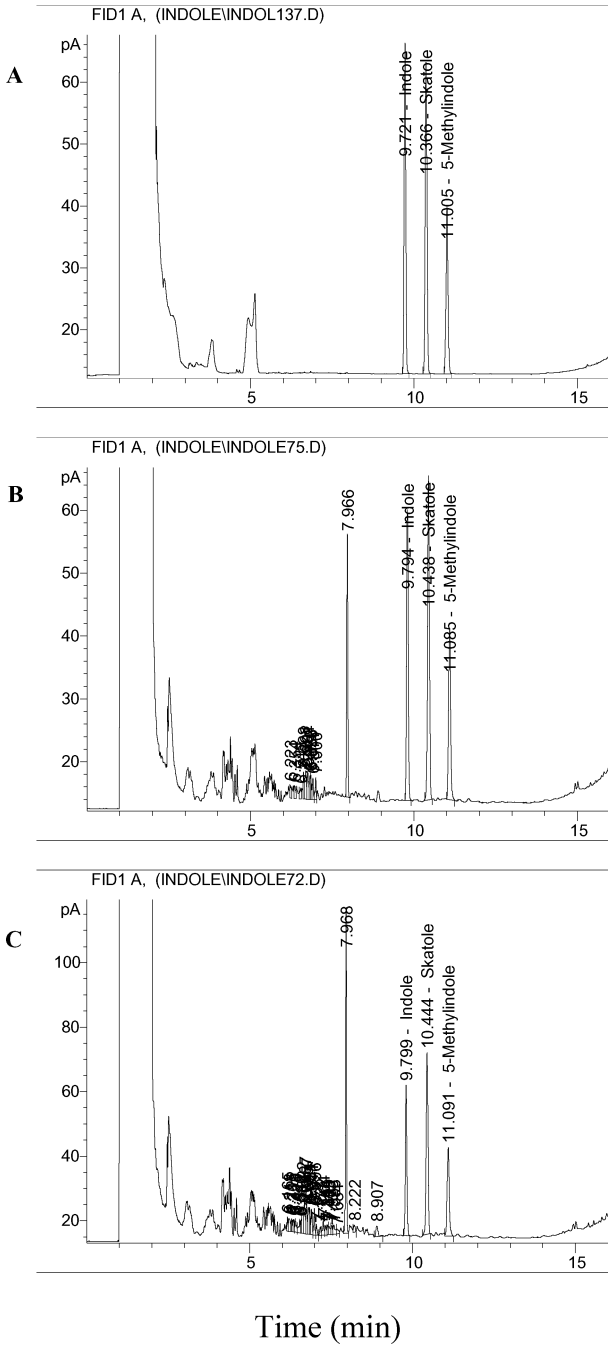

Fig. 3. Gas Chromatography (GC) tracings of (A) 10 μg mL^{-1} indole and skatole with the internal standard, 5-methylindole, in hexanes; (B) a 10 μg mL^{-1} spike of indole and skatole in water, and (C) a spike of 10 μg mL^{-1} indole and skatole into a pig fecal slurry followed by the described extraction and chromatography methods.

Table 1. Recovery of 10 μg mL^{-1} indole and skatole spikes in water and control swine fecal slurries.*

	10 μg mL^{-1} spike in water (μg mL^{-1})†	*% recovery*	*10 μg mL^{-1} spike in fecal slurries (μg mL^{-1})*†	*% recovery*
Indole	7.85 ± 0.22	78.5	7.61 ± 0.15	76.1
Skatole	9.60 ± 0.31	96.0	8.58 ± 0.18‡	85.8

*The initial concentration of indole and skatole in the control swine fecal slurry was <0.5 and 1.66 ± 0.05 μg mL^{-1}, respectively.
†The result is the mean of 4 spiked samples.
‡The value for the initial amount of skatole in the control swine fecal slurry was subtracted from the observed skatole values.

to a 10 μg mL^{-1} hexanes solution of indole, skatole, and the ISD (6 μg mL^{-1}) (Fig. 3). Recovery of 10 μg mL^{-1} spikes of indole and skatole from water was 78.5% and 96.0%, respectively, and recovery from fecal slurries was 76.1% and 85.8%, respectively (Table 1).

The effect of nitroethane and nitroethanol on the production of indole and skatole in swine fecal slurries over a 24 h period is shown in Fig. 4. At 24 h, the level of skatole (Fig. 4B) was significantly lower than the control. Also, the nitroethane and nitroethanol treatment produced less skatole at 6 h than the control. In these slurries both the nitroethane and nitroethanol additions increased the levels of indole at 6 h (Fig. 4A), and the indole level in the nitroethane treatment continued to be elevated at 24 h.

The addition of L-tryptophan to fecal slurries resulted in a large increase of indole and skatole production, 2578% and 104%, respectively, at 6 h, and 1314% and 204%, respectively, at 24 h. However, addition of nitroethane to the L-tryptophan containing mixtures resulted in a decrease of 14.9% indole and 69.0% skatole at 6 h, and a decrease of 23.5% skatole at 24 h (Fig. 5). But, indole increased by 81.1% at 24 h (Fig. 5A). The effect of nitroethanol on L-tryptophan metabolism after 6 and 24 h incubation resulted in a decrease of 8.2% indole and 41.2% skatole at 6 h and a decrease of 8.8% skatole at 24 h (Fig. 5). Again, indole levels increased by 72.2% at 24 h (Fig. 5A). It appears that the addition of both nitroethane and nitroethanol to L-tryptophan-supplemented fecal slurries decreased the production of indole and skatole at 6 h, and decreased skatole production at 24 h (Fig. 5). Even though the addition of nitroethane and nitroethanol did not eliminate the production of skatole in L-tryptophan-supplemented fecal slurries, nitroethane decreased the production of skatole ($P = 0.02$) after 6 h incubation. At 24 h the production of skatole (Fig. 5B) was still somewhat inhibited by both nitroethane and nitroethanol, while the production of indole significantly exceeded the L-tryptophan control values for indole (Fig. 5A).

The type and amount of carbohydrate source has a substantial effect on nitrogen metabolism and may influence the synthesis of indoles.[65] It was previously shown that addition of fructooligosaccharide to L-tryptophan-supplemented swine fecal slurries caused a significant decrease in skatole production and a marginal increase in the rate of indole production.[66] The researchers hypothesized this resulted from a shift in microbial metabolism due to an increased need for amino acids. In our experiments, nitroethane and nitroethanol also appeared to cause a shift in microbial metabolism.

The metabolism of L-tryptophan in bacteria occurs through several pathways.[67] L-Tryptophan can be metabolized to indole-3-acetamide or indole-3-acetaldehyde, both of which can be further metabolized to indoleacetic acid (IAA), and skatole can be produced from IAA.[17] But L-tryptophan can be converted to other products, too (e.g., *N*-formyl-kynurenine, indole-3-pyruvate, and tryptamine).

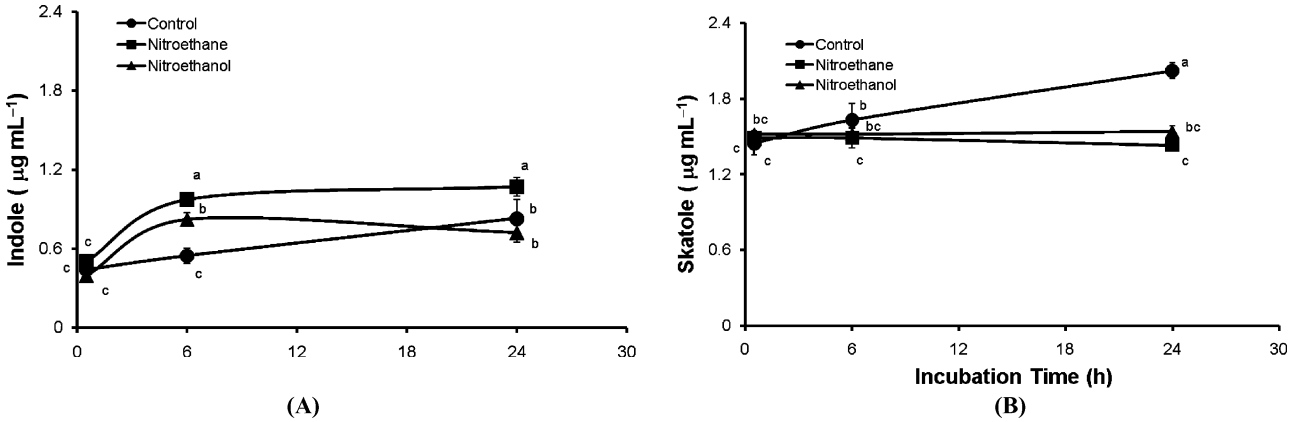

Fig. 4. Concentration of indole (A) and skatole (B) in swine fecal slurries with the addition of nitroethane and nitroethanol compared to the control over a 24 h period.

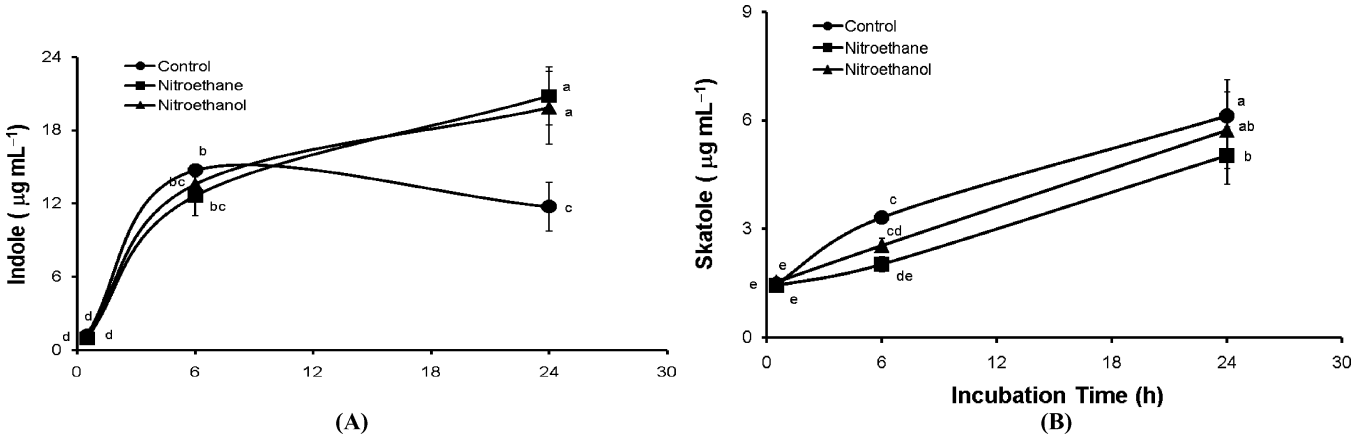

Fig. 5. Concentration of indole (A) and skatole (B) in swine fecal slurries supplemented with L-tryptophan with addition of nitroethane and nitroethanol compared to the control over a 24 h period.

Indole-3-pyruvate can be further metabolized to form IAA.[67] However, only a single metabolism step is required for the production of indole from L-tryptophan in bacteria.[67] Little is known concerning the metabolism of L-tryptophan to skatole in feces, but in the rumen L-tryptophan is metabolized by at least two different pathways to produce either indole, by the single step conversion mentioned earlier, or skatole.[68] *Lactobacillus* sp. were isolated from rumen that could produce skatole from IAA.[13] These *Lactobacillus* sp. could not produce indole, IAA, or skatole directly from L-tryptophan. The ability of ruminal *Lactobacillus* sp. to produce skatole from IAA appeared to be metabolically unique.[69] It was communicated that IAA could be converted to skatole in a variety of animal fecal microflora, including pigs and humans.[69,14] Skatole inhibited the growth of and was toxic to the *Lactobacillus* sp. that produced it and was a non-competitive inhibitor of the skatole-forming enzyme.[70] Also, some rumen bacteria can produce L-tryptophan from IAA.[71] Therefore, since L-tryptophan can be formed from intermeadiates like IAA,[71] the inhibition of skatole production may result in more L-tryptophan being available for indole production. We hypothesize that nitroethane and nitroethanol are inhibiting the pathway from L-typtophan to skatole; therefore, allowing the indole pathway to proceed. The question remains, what reason or benefit does the production of skatole have for the bacteria?[72]

Conclusions

The analytical method presented here for the quantification of indole and skatole in swine fecal slurries is a simple and quick method for determining the levels of these chemicals. Modifications to the method of Jensen and Jensen[62] by the addition of saline instead of water during the extraction process, use of hexanes (68–70°C fraction) for extraction and injection solvent, and decreasing the injector temperature to 250°C improved the FID response, which allowed the modified method to be successful. The improved method eliminates the large amount of indole and skatole decomposition in the injector. The new improved method resulted in a recovery of 78.5% indole and 96.0% skatole from water, and a recovery of 76.1% indole and 85.8% skatole from fecal slurries. These results suggest that perhaps indole and skatole are binding or reacting with a fecal slurry component(s).

This work demonstrates that a gas chromatographic FID detector can be used to quantify indole and skatole, and that certain additives like nitroethane and nitroethanol can alter the production of indole and skatole from bacteria in swine fecal slurries and from L-tryptophan-supplemented swine fecal slurries. Nitroethane and nitroethanol reduced the level of skatole in swine fecal slurries and tended to decrease the level of skatole in L-tryptophan-supplemented swine fecal slurries. The levels of indole significantly increased in L-tryptophan-supplemented swine fecal slurries upon addition of either nitroethane or nitroethanol. The level of indole also increased at 6 and 24 h when nitroethane was added to swine fecal slurries. Nitroethanol also increased the level of indole over the control at 6 h. Suggesting that addition of nitroethane or nitroethanol may be inhibiting the pathway from L-tryptophan to skatole, allowing the indole pathway to proceed. Based on this work, further studies can be made to evaluate inhibitors of the production of indole and skatole in fecal slurries. Due to the lung Clara cell toxic effects caused by skatole, the reduction or elimination of skatole production and accumulation in animal waste storage pits, waste holding reservoirs or lagoons may be beneficial to farm workers and especially to swine confinement workers.

Acknowledgments

We thank Jackie Kotzur for technical assistance. This work was funded by the United States Department of Agriculture, Agricultural Research Service.

References

[1] Mackie, R.I.; Stroot, P.G.; Varel, V.H. Biochemical identification and biological origin of key odor components in livestock waste. J. Anim. Sci. **1998**, *76*, 1331–1342.

[2] Hansen, M.N.; Kai, P.; Møller, H.B. Effects of anaerobic digestion and separation of pig slurry on odor emission. Appl. Eng. Agric. **2006**, *22*, 135–139.

[3] Hobbs, P.J.; Misselbrook, T.H.; Pain, B.F. Assessment of odours from livestock wastes by a photoionization detector, an electronic nose, olfactometry and gas chromatography-mass spectrometry. J. Agric. Engng. Res. **1995**, *60*, 137–144.

[4] Blunden, J.; Aneja, V.P. Characterizing ammonia and hydrogen sulfide emissions from a swine waste treatment lagoon in North Carolina. Atmos. Environ. **2008**, *42*, 3277–3290.

[5] Hobbs, P.J.; Misselbrook, T.H.; Pain, B.F. Emission rates of odorous compounds from pig slurries. J. Sci. Food Agric. **1998**, *77*, 341–348.

[6] Hansson, K.-E.; Lundström, K.; Fjelkner-Modig, S.; Persson, J. The importance of androstenone and skatole for boar taint. Swedish J. Agric. Res. **1980**, *10*, 167–173.

[7] Bonneau, M. Compounds responsible for boar taint, with special emphasis on androstenone: A review. Livest. Prod. Sci. **1982**, *9*, 687–705.

[8] Lundström, K.; Malmfors, B.; Malmfors, G.; Stern, S.; Petersson, H.; Mortensen, A.B.; Sørensen, S.E. Skatole, androstenone and taint in boars fed two different diets. Livest. Prod. Sci. **1988**, *18*, 55–67.

[9] Babol, J.; Squires, E.J.; Lundström, K. Hepatic metabolism of skatole in pigs by cytochrome P4502E1. J. Anim. Sci. **1998**, *76*, 822–828.

[10] Babol, J.; Squires, E.J.; Lundström, K. Relationship between metabolism of androstenone and skatole in intact male pigs. J. Anim. Sci. **1999**, *77*, 84–92.

[11] Doran, E.; Whittington, F.M.; Wood, J.D.; McGivan, J.D. Characterisation of androstenone metabolism in pig liver microsomes. Chem.-Biol. Interact. **2004**, *147*, 141–149.

[12] Macfarlane, S.; Macfarlane, G.T. Proteolysis and amino acid fermentation. In *Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology*; Gibson, G.R., Macfarlane, G.T., Eds.; CRC Press: Boca Raton, FL, USA, 1995; 75–100.

[13] Yokoyama, M.T.; Carlson, J.R.; Holdeman, L.V. Isolation and characteristics of a skatole-producing *Lactobacillus* sp. from the bovine rumen. Appl. Environ. Microbiol. **1977**, *34*, 837–842.

[14] Yokoyama, M.T.; Carlson, J.R. Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. Am. J. Clin. Nutr. **1979**, *32*, 173–178.

[15] Dickinson, E.O.; Spencer, G.R.; Gorham, J.R. Experimental induction of an acute respiratory syndrome in cattle resembling bovine pulmonary emphysema. Vet. Rec. **1967**, *80*, 487–489.

[16] Carlson, J.R.; Dyer, I.A.; Johnson, R.J. Tryptophan-induced interstitial pulmonary emphysema in cattle. Am. J. Vet. Res. **1968**, *29*, 1983–1989.

[17] Yokoyama, M.T.; Carlson, J.R. Dissimilation of tryptophan and related indolic compounds by ruminal microorganisms in vitro. Appl. Microbiol. **1974**, *27*, 540–548.

[18] Yokoyama, M.T.; Carlson, J.R.; Dickinson, E.O. Ruminal and plasma concentrations of 3-methylindole associated with tryptophan-induced pulmonary edema and emphysema in cattle. Am. J. Vet. Res. **1975**, *36*, 1349–1352.

[19] Carlson, J.R.; Dickinson, E.O.; Yokoyama, M.T.; Bradley, B. Pulmonary edema and emphysema in cattle after intraruminal and intravenous administration of 3-methylindole. Am. J. Vet. Res. **1975**, *36*, 1341–1347.

[20] Carlson, J.R.; Yokoyama, M.T.; Dickinson, E.O. Induction of pulmonary edema and emphysema in cattle and goats with 3-methylindole. Science **1972**, *176*, 298–299.

[21] Bradley, B.J.; Carlson, J.R. Ultrastructural pulmonary changes induced by intravenously administered 3-methylindole in goats. Am. J. Pathol. **1980**, *99*, 551–560.

[22] Huang, T.W.; Carlson, J.R.; Bray, T.M.; Bradley, B.J. 3-Methylindole-induced pulmonary injury in goats. Am. J. Pathol. **1977**, *87*, 647–666.

[23] Bradley, B.J.; Carlson, J.R.; Dickinson, E.O. 3-Methylindole-induced pulmonary edema and emphysema in sheep. Am. J. Vet. Res. **1978**, *39*, 1355–1358.

[24] Turk, M.A.; Breeze, R.G.; Gallina, A.M. Pathologic changes in 3-methylindole-induced equine bronchiolitis. Am. J. Pathol. **1983**, *110*, 209–218.

[25] Adams, Jr., J.D.; Laegreid, W.W.; Huijzer, J.C.; Hayman, C.; Yost, G.S. Pathology and glutathione status in 3-methylindole-treated rodents. Res. Commun. Chem. Pathol. Pharmacol. **1988**, *60*, 323–336.

[26] Turk, M.A.M.; Flory, W.; Henk, W.G. Chemical modulation of 3-methylindole toxicosis in mice: Effect on bronchiolar and olfactory mucosal injury. Vet. Pathol. **1986**, *23*, 563–570.

[27] Setzer, A.K.; Slotnick, B. Disruption of axonal transport from olfactory epithelium by 3-methylindole. Physiol. Behav. **1998**, *65*, 479–487.

[28] Slotnick, B.; Bodyak, N. Odor discrimination and odor quality perception in rats with disruption of connections between the olfactory epithelium and olfactory bulbs. J. Neurosci. **2002**, *22*, 4205–4216.

[29] Slotnick, B. Olfactory performance of rats after selective deafferentation of the olfactory bulb by 3-methyl indole. Chem. Senses **2007**, *32*, 173–181.

[30] Kubow, S.; Dubose, Jr.; C.M.; Janzen, E.G.; Carlson, J.R.; Bray, T.M. The spin-trapping of enzymatically and chemically catalyzed free radicals from indolic compounds. Biochem. Biophys. Res. Commun. **1983**, *114*, 168–174.

[31] Huijzer, J.C.; Adams, Jr., J.D.; Jaw, J.-Y.; Yost, G.S. Inhibition of 3-methylindole bioactivation by the cytochrome P-450 suicide substrates 1-aminobenotriazole and α-methylbenzylaminobenzotriazole. Drug Metab. Dispos. **1989**, *17*, 37–42.

[32] Thornton-Manning, J.; Appleton, M.L.; Gonzalez, F.J.; Yost, G.S. Metabolism of 3-methylindole by vaccinia-expressed P450 enzymes: Correlation of 3-methyleneindolenine formation and protein-binding. J. Pharmacol. Exper. Therap. **1996**, *276*, 21–29.

[33] Ruangyuttikarn, W.; Skiles, G.L.; Yost, G.S. Identification of a cysteinyl adduct of oxidized 3-methylindole from goat lung and human liver microsomal proteins. Chem. Res. Toxicol. **1992**, *5*, 713–719.

[34] Boyd, M.R.; Witschi, H.P. Biochemical mechanisms in chemical-induced lung injury: Roles of metabolic activation. Crit. Rev. Toxicol. **1980**, *7*, 103–176.

[35] Beier, R.C. Role of metabolism and the toxic response of some naturally occurring chemicals in food. In *The Mammalian Metabolism of Agrochemicals*, Hutson, D.H., Paulson, G.D., Eds.; *Progress in Pesticide Biochemistry and Toxicology*; John Wiley & Sons Ltd.: Chichester, England, **1995**, Vol. 8, 309–332.

[36] Beier, R.C.; Norman, J.O. The toxic factor in white snakeroot: Identity, analysis and prevention. Vet. Human Toxicol. **1990**, *32(Suppl)*, 81–88.

[37] Beier, R.C.; Norman, J.O.; Irvin, T.R.; Witzel, D.A. Microsomal activation of constituents of white snakeroot (*Eupatorium rugosum* Houtt) to form toxic products. Am. J. Vet. Res. **1987**, *48*, 583–585.

[38] Beier, R.C.; Norman, J.O.; Reagor, J.C.; Rees, M.S.; Mundy, B.P. Isolation of the major component in white snakeroot that is toxic after microsomal activation: Possible explanation of sporadic toxicity of white snakeroot plants and extracts. Nat. Toxins **1993**, *1*, 286–293.

[39] Ruangyuttikarn, W.; Appleton, M.L.; Yost, G.S. Metabolism of 3-methylindole in human tissues. Drug Metab. Dispos. **1991**, *19*, 977–984.

[40] Nichols, W.K.; Mehta, R.; Skordos, K.; Macé, K.; Pfeifer, A.M.A.; Carr, B.A.; Minko, T.; Burchiel, S.W.; Yost, G.S. 3-Methylindole-induced toxicity to human bronchial epithelial cell lines. Toxicol. Sci. **2003**, *71*, 229–236.

[41] Kowalewska, J.; Zelazowska, H.; Babuchowski, A.; Hammond, E.G.; Glatz, B.A.; Ross, F. Isolation of aroma-bearing material from *Lactobacillus helveticus* culture and cheese. J. Dairy Sci. **1985**, *68*, 2165–2171.

[42] Rodgman, A.; Cook, L.C. The composition of cigarette smoke. XI. Heterocyclic nitrogen compounds from Turkish tobacco smoke. Tobacco Sci. **1962**, *6*, 176–179.

[43] Hoffmann, D.; Rubin, J. Chemical studies on tobacco smoke. 1. The quantitative determination of indoles in cigarette smoke. Beitr. Tabakforsch. **1966**, *3*, 409–414.

[44] Wynder, E.L.; Hoffmann, D. *Tobacco and Tobacco Smoke: Studies in Experimental Carcinogenesis*. Academic Press: New York, 1967, 371–379.

[45] Hoffmann, D.; Rathkamp, G. Quantitative determination of 1-alkylindoles in cigarette smoke. Anal. Chem. **1970**, *42*, 366–370.

[46] Hoshika, Y. Simultaneous gas chromatographic analysis of lower fatty acids, phenols and indoles using a glass capillary column. J. Chromatogr. **1977**, *144*, 181–189.

[47] Silwar, R.; Kamperschröer, H.; Tressl, R. Gaschromatographisch-massenspektrometrische untersuchungen des röst-kaffeearomas – quantitative bestimmung wasserdampfflüchtiger aroma-stoffe. Chem. Mikrobiol. Technol. Lebensm. **1987**, *10*, 176–187.

[48] Schulz, H. Bestimmung von indol und skatol in meerestieren durch hochleistungs-flüssigchromatographie (HPLC). Z. Lebensm.-Unters. Forsch. **1986**, *183*, 331–334.

[49] Thornton-Manning, J.R.; Ruangyuttikarn, W.; Gonzalez, F.J.; Yost, G.S. Metabolic activation of the pneumotoxin, 3-methylindole, by vaccinia-expressed cytochrome P450s. Biochem. Biophys. Res. Commun. **1991**, *181*, 100–107.

[50] Donham, K.J.; Merchant, J.A.; Lassise, D.; Popendorf, W.J.; Burmeister, L.F. Preventing respiratory disease in swine confinement workers: Intervention through applied epidemiology, education, and consultation. Am. J. Ind. Med. **1990**, *18*, 241–261.

[51] Thu, K.; Donham, K.; Ziegenhorn, R.; Reynolds, S.; Thorne, P.S.; Subramanian, P.; Whitten, P.; Stookesberry, J. A control study of the physical and mental health of residents living near a large-scale swine operation. J. Agric. Safety Health **1997**, *3*, 13–26.

[52] Schiffman, S.S. Livestock odors: Implications for human health and well-being. J. Anim. Sci. **1998**, *76*, 1343–1355.

[53] Peleran, J.C.; Bories, G.F. Gas chromatographic determination and mass spectrometric confirmation of traces of indole and 3-methylindole (skatole) in pig back fat. J. Chromatogr. **1985**, *324*, 469–474.

[54] Porter, M.G.; Hawe, S.M.; Walker, N. Method for the determination of indole and skatole in pig fat. J. Sci. Food Agric. **1989**, *49*, 203–209.

[55] Hansen-Møller, J. Determination of indolic compounds in pig back fat by solid-phase extraction and gradient high-performance liquid chromatography with special emphasis on the boar taint compound skatole. J. Chromatogr. **1992**, *624*, 479–490.

[56] Mortensen, A.B.; Sørensen, S.E. Relationship between boar taint and skatole determined with a new analysis method. In *Proceedings of the 30*th *European Meeting of Meat Research Workers*, September 9–14, 1984, Bristol, UK; 394–396.

[57] Wilkins, C.K. Analysis of indole and skatole in porcine gut contents. Int. J. Food Sci. Technol. 1990, 25, 313–317.

[58] Dehnhard, M.; Bernal-Barragan, H.; Claus, R. Rapid and accurate high-performance liquid chromatographic method for the determination of 3-methylindole (skatole) in faeces of various species. J. Chromatogr. **1991**, *566*, 101–107.

[59] Yasuhara, A. Identification of volatile compounds in poultry manure by gas chromatography-mass spectrometry. J. Chromatogr. **1987**, *387*, 371–378.

[60] Loughrin, J.H.; Szogi, A.A.; Vanotti, M.B. Reduction of malodorous compounds from a treated swine anaerobic lagoon. J. Environ. Qual. **2006**, *35*, 194–199.

[61] Bradley, B.J.; Carlson, J.R. A gas–liquid chromatographic procedure for the determination of indole and 3-methylindole in bovine plasma. Anal. Biochem. **1974**, *59*, 214–219.

[62] Jensen, M.T.; Jensen, B.B. Gas chromatographic determination of indole and 3-methylindole (skatole) in bacterial culture media, intestinal contents and faeces. J. Chromatogr. B **1994**, *655*, 275–280.

[63] Jensen, M.T.; Cox, R.P.; Jensen, B.B. 3-Methylindole (skatole) and indole production by mixed populations of pig fecal bacteria. Appl. Environ. Microbiol. **1995**, *61*, 3180–3184.

[64] Bryant, M.P.; Burkey, L.A. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. **1953**, *36*, 205–217.

[65] Hawe, S.M.; Walker, N.; Moss, B.W. The effects of dietary fibre, lactose and antibiotic on the levels of skatole and indole in faeces and subcutaneous fat in growing pigs. Anim. Prod. **1992**, *54*, 413–419.

[66] Xu, Z.-R.; Hu, C.-H.; Wang, M.-Q. Effects of fructooligosaccharide on conversion of L-tryptophan to skatole and indole by mixed populations of pig fecal bacteria. J. Gen. Appl. Microbiol. **2002**, *48*, 83–89.

[67] Ummadi, M.; Weimer, B.C. Tryptophan catabolism in *Brevibacterium linens* as a potential cheese flavor adjunct. J. Dairy Sci. **2001**, *84*, 1773–1782.

[68] Claus, R.; Weiler, U.; Herzog, A. Physiological aspects of androstenone and skatole formation in the boar—A review with experimental data. Meat Sci. **1994**, *38*, 289–305.

[69] Yokoyama, M.T.; Carlson, J.R. Production of skatole and *para*-cresol by a rumen *Lactobacillus* sp. Appl. Environ. Microbiol. **1981**, *41*, 71–76.

[70] Honeyfield, D.C.; Carlson, J.R. Assay for the enzymatic conversion of indoleacetic acid to 3-methylindole in a ruminal *Lactobacillus* species. Appl. Environ. Microbiol. **1990**, *56*, 724–729.

[71] Allison, M.J.; Robinson, I.M.; Baetz, A.L. Tryptophan biosynthesis from indole-3-acetic acid by anaerobic bacteria from the rumen. J. Bacteriol. **1974**, *117*, 175–180.

[72] Honeyfield, D.C.; Carlson, J.R. Effect of indoleacetic acid and related indoles on *Lactobacillus* sp. strain 11201 growth, indoleacetic acid catabolism, and 3-methylindole formation. Appl. Environ. Microbiol. **1990**, *56*, 1373–1377.